TITLE "Good to Great" **PROJECT**

Continued from page

Goals for Gold

Wiki — clean, easy to navigate, subpages, nice color scheme, thematic, graphics, animation

Presentation — charismatic, diagrams/visuals > words, limited interaction

Human Practices — multidisciplinary, multilayered, interaction with other teams, visit schools, distribute pamphlets @ library, iGEM-sponsored tutoring, summer camp, ASF, NGF, local charities, create curriculum, social media

Poster — thematic, subtle graphics, color scheme, flow, well-organized

Science — mathematical & graphical models, repeatability, functional prototype, results, larger impacts

Meeting

- compared William & Mary and TAS Taipei
  - ↳ rubric for each part of the project
- made our Goals for Gold

HW — Pre-Lab for a Transformation using PUC19 (due Thursday morning)

- Title
- Purpose
- Background → 7-9 sentences
- Materials
- Methods
- Pre-Lab ? → what would a successful transformation look like

Continued to page

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DATE 12/10/2015

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PROJECT

TITLE NEB 10-beta High Efficiency Transformation (PUC19)

Continued from page

Purpose: The objective of a transformation is to make multiple copies of DNA, once they are manipulated. It will then be transferred into bacteria, specifically E. coli.

Background Information: In a transformation, circular pieces of DNA called plasmids are manipulated. In biotechnology, plasmids can be easily engineered and transferred into bacteria to see its effects. Sterile techniques are used in this lab to prevent contamination of the lab space and the data. Specifically for this transformation, pUC19 will serve as a plasmid cloning vector in E. coli, and this is how DNA will make several copies. After these copies are made, the cells will be grown on agar plates. The agar serves as food to fuel the cells with energy so that they can divide. LB broth helps the cells to recover and grow. The cells are incubated at 37°C because that is their optimal temperature.

Materials: NEB 10-beta E. coli cells
ice
micropipettes, tips
waste beaker
transformation tubes
plasmid DNA
room temperature SOC
centrifuge
Amp plates
incubator

Protocol:

1) Thaw a tube of NEB* 10-beta E. coli cells ~~!!!!!~~ for 10 minutes or until the ice crystals disappear. Mix gently and pipette 50 µL into a transformation tube on ice.
2) Add 1-5 µL of plasmid DNA containing 1 pg-100 ng to the cell mixture. Flick 4-5 times but do not vortex. by PUC19 Add 1 µL of dH_2O to control (no plasmid).
3) Place mixture on ice for 30 minutes.
4) Heat shock at 42°C for 30 seconds.
5) Place on ice for 5 minutes.
6) Pipette 950 µL of room temperature SOC into the mixtures.
7) Place at 37°C (incubator) for 60 minutes. Shake at 250 rpm or rotate, using a centrifuge.

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TITLE NEB 10-Beta High Efficiency Transformation PROJECT

Continued from page 2

8) Warm selection plates to 37°C.
9) Flick the tube and invert to mix the cells. Make 10-fold dilutions in SOC.
10) Spread 50-100 µL of each dilution onto an Amp plate. Incubate overnight at 37°C or incubate at 30°C for 24-36 hours or 25°C for 48 hours.

Pre-Lab Question:
1) What would a successful transformation look like?
Multiple colonies of about the same size will form. They will be distributed relatively evenly throughout the plate. When the DNA is put into gel electrophoresis, results will show that the specific sequence is present in the plasmid. Control?

~~Pla~~ Labeling: EC 5 ○ ○ - no plasmid
LH 5 + + - plasmid - plain
NC 5 ⊕ ⊕ - plasmid - Amp

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PROJECT

TITLE Transformation Results

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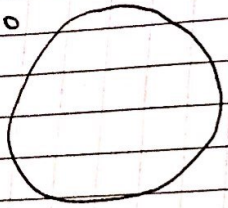
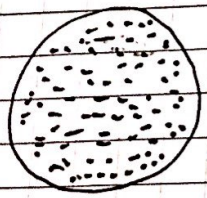
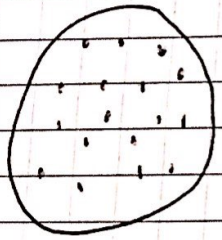
0 

no plasmid/Amp
negative control
no growth

+ 

NEB 10
plain plate with plasmid
a lot of colonies → both with and without plasmids

+ 

NEB 10
Amp plate with plasmid
less colonies → can calculate transformation efficiency

Future Experiments

- find a single colony → inoculate it (collect part of the colony) → streak → liquid colony → etc.

- decrease/dilute to have less colonies

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DATE 1/7/2016

TITLE Potential Projects – Brainstorm/ Questions PROJECT

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Zinc Biosensor Questions:

1) Does the biosensor detect outside of 8-15 μM?
2) what do you envision about our iGEM team?
3) would it be more helpful to engineer a pathway with standard systems rather than using a zinc biosensor?
   ↳ proof of concept for precision vs. dynamic engineering?
4) Can you provide an analogy to clarify the differences between precision and dynamic engineering?
5) Could you clarify which colors correlate to which levels of Zinc? There was an error in the press release.
6) Is there a kill switch?
7) How exclusive is the biosensor to Zinc?

Notes – Skype Interview

#6 → no killswitch for the system, but precision metabolic engineering can be used to create one

- degradation tags → adding deg tags affect how quickly you can move from one state to another
- developing a biosensor →
  - need something that can sense whatever is needed to be identified
    ex/ transcriptional regulatory mechanism/quorum sensing
  - challenges:
    ① sensing mechanism must be exclusive to only what you want to detect
    ② time (sims require ~24 hrs to obtain desired result)
      ↳ possible solution – use of catalysts, especially pigment producing enzymes
        - ideal catalyst would be one that can expedite transcription
  - need a well-designed, elegant construct
- inducer → a chemical that binds to a molecule and invokes a certain behavior
  ↳ standard inducible promoters → chromoproteins → ex/ TS Purple
    ex/ LacI

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DATE 1/21/2016

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TITLE Chromoproteins/RBS/Degradation tags Research

Continued from page

- Reporters → [in syn bio] mechanism to display information
  ex) If you design a biosensor system that is sensitive to substrate A, then it would be beneficial to have a way of displaying results rather than performing additional assays
  - fluorescent proteins → cells fluoresce when excited by light of a certain wavelength
  - luciferases → enzymes that cause a cell to catalyze a reaction that produces light
- Types of reporters →
  ① chromoproteins
  ② fluorescent proteins (i.e. GFP/RFP)
  ③ luciferase proteins
  ④ enzymes that produce colored substrates
- Different ways of measuring/quantifying reporters
  ↳ depends on particular reporter and type of characterization data desired
  ① microscopy → useful for obtaining spatial and temporal information, especially at a single cell level
  ② flow cytometers → measuring distribution in reporter activity across a large population of cells
  ③ plate readers → population average measurements of many different samples over time
- Chromoproteins → responsible for coloration in corals/sea anemones
  - expressed pigment can be seen with the naked eye
  - collection of reporter genes
  - function → quantify the level of induction and amount of protein produced
    ex) Ts Purple, ScOrange, aSpink
- RBS = Ribosomal binding site → RNA sequence found in mRNA which ribosomes can bind and initiate translation
  - translation in bacteria must be initiated by both an RBS sequence and a start codon
    ↳ protein coding sequence begins with start codon in iGEM registry
    ↳ if you want to build a BioBrick system that produces a protein, you need to select an RBS part and put it upstream of the protein coding sequence to be translated

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DATE 2/4/2014

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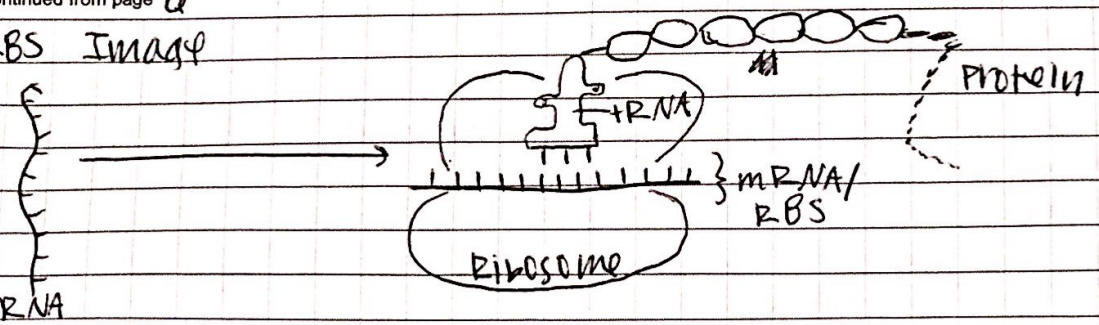
TITLE Chromoproteins/RBS/Degradation Tags Research PROJECT

Continued from page 6

- RBS Image



- Degradation Tags → short polypeptide sequence that marks a protein for degradation by the cell's protein recycling machinery
  - effectively decreases the protein's half life or time that a protein will exist in a cell
- constructs
  - Three main repressible/inducibly promoter systems (rep-proms):
    1. LacI-pLac
    2. TetR-pTet
    3. AraC-pBad

    } all found in the iGEM registry
  - Two different ways to approach construct designs:
    1. Production fully expressed in "off" state then turned "on" to a specific level in addition to an inducer
       - have a chromoprotein under control of an inducible protein, study/measure the steady state induced level of production, measure how protein is produced
       - changing RBS from strong to weak → ↓ amount of protein, ↑ time required to produce
       - adding degradation tags
       - main thing to characterize → how different modifications (RBS) and protein deg tags change levels of induction
    2. Production is "on" initially then turned "off" upon addition of an inducer
       - use 2 different inducible promoters to create a switch
       - little more "tricky"

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DATE 2/4/2014

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PROJECT

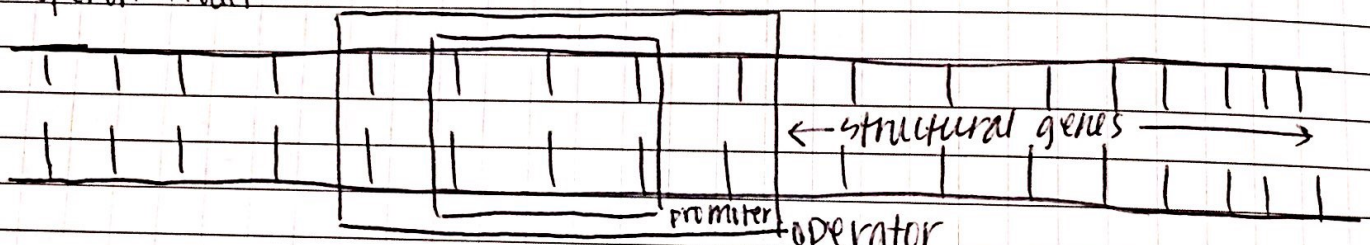
TITLE Gene Expression

Continued from page

- Gene Expression in prokaryotes (ex. lac operon)
  - how genes are turned ON or OFF
  - prokaryotes ~~are~~ have simpler gene expression than eukaryotes → few controls
  - operons: sections of DNA that consist of ≥1 genes and their controlling elements
    - middle of an operon are structural genes: → sections that code for ≥1 mRNA molecules [that code for proteins]
    - promoter region → "turns on" the prokaryotic gene; in the beginning of the operon
      - RNA pol must ~~attach~~ attach itself to the promoter to begin mRNA synthesis
    - operator region → "turns off" the while operon if a regulatory molecule (i.e. repressor) attaches at the operator
      - RNA pol is blocked from continuing down the strand

*selective gene expression – blocking/unblocking the operator – is how bacterial cells produce only certain proteins at certain times

- Operon Model:



- Gene Expression in Eukaryotes
  - no operons, but instead eukaryotes have enhancers and silencers
    - silencer → sections of DNA that decrease the expression of a gene
    - enhancer → sections of DNA that increase a gene's expression
  - Eukaryotic genes are always "turned on", but expression can be significantly increased or decreased with enhancers and silencers.
- Prokaryotic Gene Regulation of Metabolism
  1. enzyme inhibition → slows down enzymatic processes and provides effective control of the metabolic pathway
  2. regulator protein → protein that binds to the operator [region of the operon] to control their metabolic pathways overtime
     - ex. repressors

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TITLE Gel Electrophoresis Lab **PROJECT**

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Purpose: The objective of completing gel electrophoresis is to analyze DNA samples by separating bands of DNA according to their size.

Background Information: The agarose gel contains wells that the DNA/RNA samples are placed in using a micropipette. The gel has pores that allow the DNA/RNA to move towards the opposite side. The gel is put in an electric box with [TAE] buffer. The wells are on the negative (black) side, while the other end is at the positive side (red). Because DNA is negative, it will "run to red" since it is attracted to the positive charge. Longer strands take more time to maneuver through the pores, so shorter bands will travel further down the gel.

Materials:
- agarose gel
- methylene blue ~~stain~~ staining solution (60 mL)
- TAE buffer
- dH_2O
- 2 beakers (400 mL)
- digital micropipets
- electrophoresis chamber/power supply
- marker
- paper towels
- staining tray
- thermometer
- biological reagants/samples
- metric ruler
- resealable bag
- white surface/paper
- lightbox
- disposable pipet tips

Protocol:
1) Gently slide the gel into the chamber will the wells on the negative side.
2) Pour the TAE buffer on top of the gel until it is completely submerged.
3) Gently flick the tubes containing the DNA samples to mix its contents.
4) Using a micropipette, gently transfer about 20-30 µL of sample into a well. Be careful not to puncture the well.
5) Repeat step 4, and make sure a new, clean tip is used for each sample.
6) Place the lid on the chamber and turn it on by plugging the

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TITLE PROJECT

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power source to an electrical source. Make sure the wires are properly matched.
7) Run it for about 30 minutes to 2 hours. The first bands should be about 1cm from the end of the gel. Turn the power off when done.
8) Carefully take out the gel and put it on a lightbox to analyze the bands.
* If necessary, add 40 mL of methylene blue staining solution onto the gel. Allow for staining to occur for about 5 minutes before putting it on the lightbox.

TITLE Genetic Circuits PROJECT

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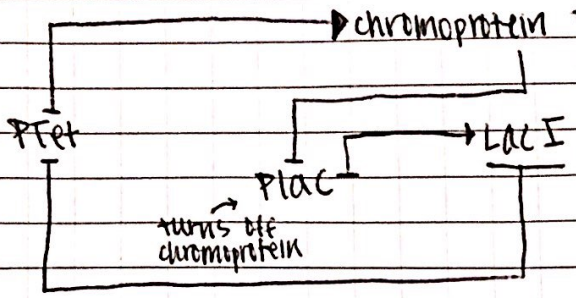
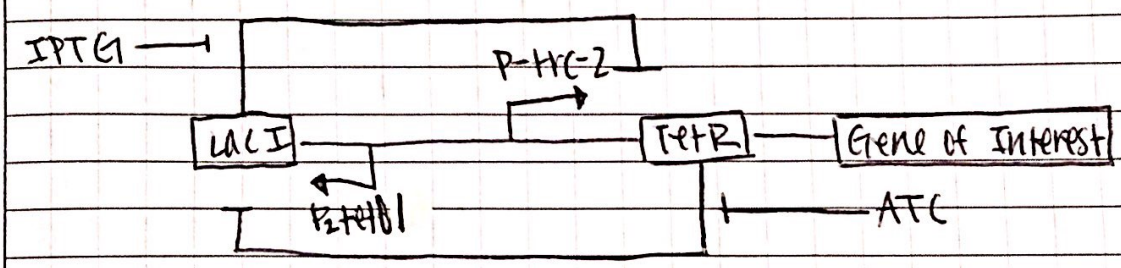


a basic toggle switch
→ circular

- The Toggle Switch → circuit consisting of two repressors
  ① TetR
  ② LacI
  } both mutually repress each other



- The LacI repressor is inhibiting the P-trc-2 promoter → repressor reporter
- The TetR repressor is inhibiting the P_2 tet O1 promoter → promoter reporter
- The ATC inducer inhibits the expression of TetR repressor, therefore promoting the expression of P_2 tet O1 promoter and the LacI repressor.
- The IPTG inducer inhibits the expression of LacI repressor, therefore promoting the expression of Ptrc-2 promoter and the gene of interest.

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DATE 3/1/2016

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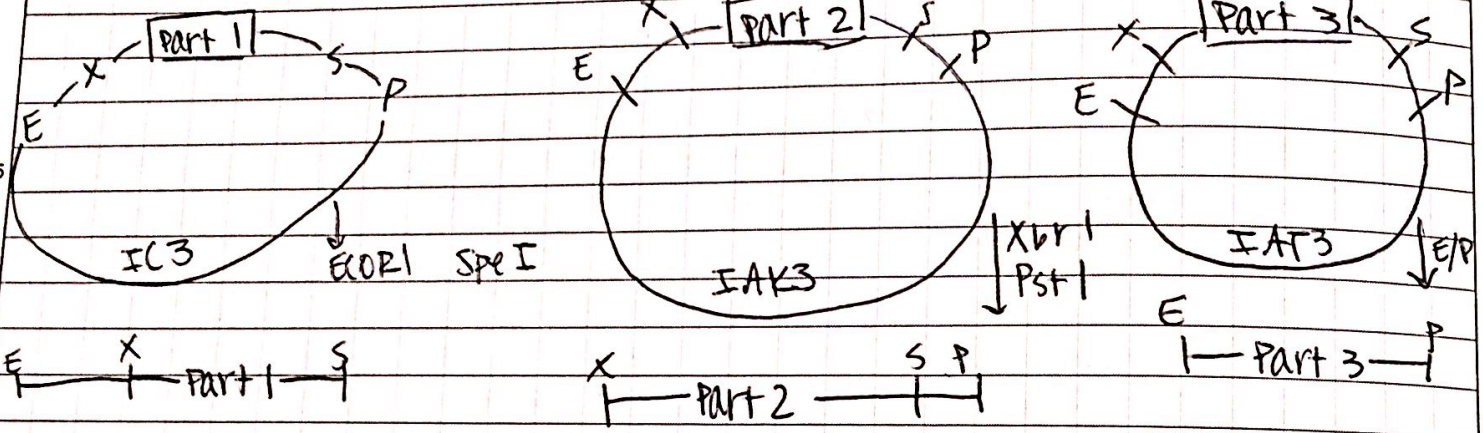
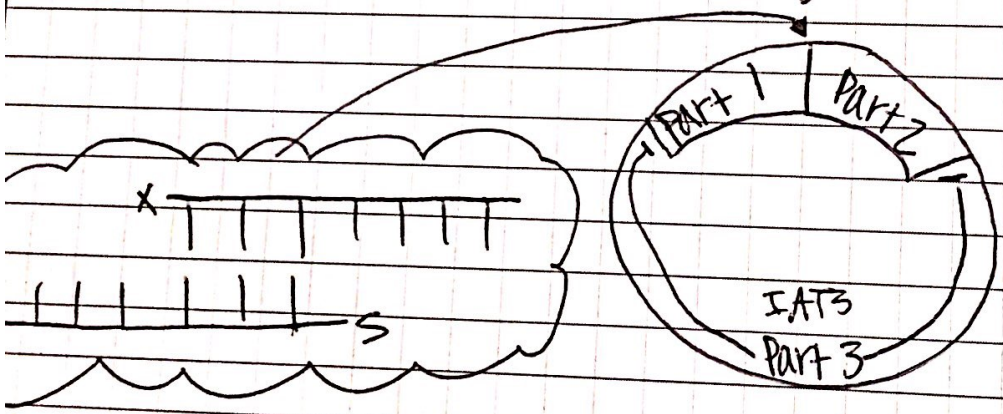
TITLE GaTech Notes on Assemblies PROJECT

Continued from page

- Types of antibiotics to use in plates: [4]
  1. Kanamycin (Kan)
  2. Chloramphenicol (Cam)
  3. Tetracycline (Tet)
  4. Ampicillin/carbenicillin (Amp/Carb)
- Types of Plasmids: [3]
  1. IC3 → Cam resistant
  2. IAK3 → Amp and Kan resistant
  3. IAT3 → Amp and Tet resistant





Golden Rule of 3A Assemblies:
1. ES
2. XP
3. EP

TITLE 7/11/2016 GT ew pLac IA3 PROJECT

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1) Mini-Prep
- 2500 xg for 5 min instead of 10,000 xg for 1 min
- once solution II is put in, do not let it sit for >5 min
- After soln III microcentrifuge for ≥13,000 mg or 17900 rcf for 10 min
- Centrifuge wash buffers for 1 min.
- step 14 - centrifuge w/ elution buffer for 1 min (not 60 s)
- not necessary to repeat last wash buffer

* step 12 → centrifuge empty mini column @ max speed for 2 min. to remove ethanol
- elution buffer - 50 µL

* vortex before nanodrop

2) Nanodrop

73.4 ng/ul

A260	1.469	
A280	0.833	
260/280	1.76	(1.8)
260/230	1.91	(2.1)

$\frac{73.4\ ng}{ul} \left| \frac{1\ ug}{1000\ ng} \right. = .0734\ ug/uL$

$13.624\ uL = 1\ ug\ DNA$

13.624 µL DNA
2. µL restriction enzyme (E)
1. µL standard enzyme (S)
+ 3.38 dH_2O
20. µL total volume

3) Digest
① water
② DNA } order
③ enzyme

* 200 band did not show up

* re-mix before non-re-mix
- 1 hr @ 37°C, 20 min at 80°C

4) Ligation
3:1 molar ratio vector:insert
digest 1 ug

	insert length (KB)	vector length	total length (KB)
vector IC3		2200	3200
IA3 part 1 (BW plac)	200	3450	3650
IA3 part 2 (CIpXP)	2000	3200	5200

~[DNA] ng/kb·mL

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total length (KB)
0.31
0.27
0.19

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TITLE 7/11/2016 GT

PROJECT

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		µL
vector	% total ℓ = 0.31 → $\frac{.31}{.27}$ ×3	1
Part 1	= 0.27	3.4
Part 2	= 0.19 → $\frac{.31}{.19}$ ×3	4.9

* actual measurements

+2 µL ligase buf
+1 µL ligase
+ 7.7 µL dH_2O
20 µL

ligase practice	length insert	length vector	total	% total ℓ	µL
vector 3T5	1000	3200	4200	0.24	1
Part 1 pXR lac I GFP DAS	1800	3400	5200	0.19	3.8 3.789
Part 2 BR11 ClpXP-cl 3T5	3000	2900	5900	0.17	4.2 4.235

+2 ligase buf
+1 ligase
⇒ 8 µL dH_2O 7.976
20 µL

: phosphatase - 1 hr. 20 min deactivation
: last = enzyme

- 1 hr
- no deactivation required
- buffer → has ATP, Aliquot to smaller amts

5) Transformation
- Thaw on ice for 5 min
- 10 µL ligation mixture into 100 mL competent cells
- flick to mix
- ice for 30 min
- 200 mL of LB
- Incubator @ 37°C for one hr

6) Plate
150 µL of cells on plate

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TITLE 7/12/2016 GT PROJECT

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① Digest of weak pLAC * (and chromoprotein)

$\frac{73.4\ ng}{1\ \mu L} \left| \frac{1\ mg}{1000\ ng} \right. = 0.0734\ mg/\mu L$ 13.62 μL = 1 μg DNA

$\frac{0.0734\ mg}{\mu L} = \frac{1\ mg}{x}$ $0.0734x = 1$

13.62 μL DNA
2 μL RE (E)
1 μL SE (S)
+3.38 μL dH_2O
20 μL total

reminder: ①water ②DNA ③enzyme
*never use the last bit of DNA

* well 1 (edge) - LH digest weak pLAC → 200 bp band still did not show up
well 2 (next to ladder) - digest XP blue chromoprotein

② Ligation of pAR and the chromoprotein

			length insert	length vector	total	~[DNA]	μL
vector		AK3	1000	~~3450~~ 3200	~~4450~~ 4200	~~0.225~~ 0.2381	1
part 1	pAR lacI	~~3C5~~ 3200	1113	3206	4313	0.232	~~2.91~~ 3.13
part 2	chromoprotein	IC3 2200	469	~~2200~~ 2100	2869	~~0.349~~ 0.3611	~~1.93~~ 2.0

2 ligase buf
1 ligase
+ 11.16 dH_2O
20 μL total

1 μL vector
2.91 μL part 1
1.93 μL part 2
2 μL ligase buffer
1 μL ligase
11.16 μL dH_2O

ACTUAL MEASUREMENTS

			insert	vector	total	~[DNA]	μL
vector		AK3	1000	3200	4200	0.2381	1
part 1	pAR lacI 3C5		1113	3200	4313	0.2319	3.13
part 2	blue chromo IC3		469	2100	2769	0.3611	~~1.93~~ 2.0

2 lig buf
1 ligase
0.87 ~~μL~~ μL H_2O

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TITLE Workflow Week 1 (8/15-8/19) **PROJECT**

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① Liquid culture (overnight) → 8/16/2016

1a and 1b: pAR LacI TS Purp DAS DH10 8/16/2016
2a and 2b: pAR LacI TS Purp AK3 DH10 8/16/2016
3a and 3b: pAR LacI TS Purp LAA 8/16/2016

② Miniprep (with Qiagen) and Nanodrop → 8/17/2016

1a= A260: 1.651
A260/A280 = 1.81 1.8
82.5 ng/μL

2a= A260: 2.257
A260/A280: 1.78 1.8
112.8 ng/μL

3a= A260: 1.952
A260/A280: 1.72 1.8
97.6 ng/μL

1b= A260: 1.434
A260/A280: 1.74 1.8
71.7 ng/μL

2b= A260: 2.226
A260/A280: 1.79 1.8
111.3 ng/μL

3b: A260: 2.285
A260/A280: 1.83 1.8
114.3 ng/μL

③ Digest (① water, ② enzymes, ③ DNA) → 8/17/16

1a= 12.20 μL DNA
2 μL RE
1 μL SE
4.80 μL dH_2O

2a= 8.87 μL DNA
2 μL RE
1 μL SE
6.13 μL dH_2O

3a= 10.25 μL DNA
2 μL RE
1 μL SE
6.75 μL dH_2O

1b= 13.95 μL DNA
2 μL RE
1 μL SE
3.05 μL dH_2O

2b= 8.98 μL DNA
2 μL RE
1 μL SE
8.02 μL dH_2O

3b= 8.75 μL DNA
2 μL RE
1 μL SE
8.25 μL dH_2O

Results from Gel (8/18/16) → bands matched up at correct band lengths

④ Ligation → 8/18/16 (only LAA & DAS)

Part 1: pAR RBS LacI TS purple AK3
Part 2: R11 ClpXP CI AK3
Vector: IC3

Insert ℓ	vector ℓ	Total ℓ	~[DNA]	μL
690	3200	3890	0.257	3.8
2974	3200	6174	0.162	6
1000	2100	3100	0.323	1

2 ligase buffer
1 ligase
+6.2 dH_2O
20 μL rxn

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PROJECT

TITLE

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	Insert ℓ	Vector ℓ	Total	~[DNA]	µL
Part 1: pAR ~~[illegible]~~ RBS LacI TS Purp DAS AK3	921	3200	4121	0.243	4
Part 2: R11 ClpXP CI AK3	2974	3200	6174	0.162	6
Vector: IC3	1000	2100	3100	0.323	1
					2 ligase buff
					1 ligase
					+ ~~[illegible]~~ µL dH_2O
					20 µL rxn

	Insert ℓ	Vector ℓ	Total	~[DNA]	µL
Part 1: pAR RBS LacI TS Purp LAA AK3	920	3200	4120	0.243	4
Part 2: R11 ClpXP CI AK3	2974	3200	6174	0.162	6
Vector: IC3	1000	2100	3100	0.323	1
					2 ligase buff
					1 ligase
					+ ~~[illegible]~~ µL dH_2O
					20 µL rxn

(5) Transformation → LAA & DAS → 8/18/2016 (includes plates)

1) 8/18/2016 pAR LacI TS Purp DAS AK3 DH10 iGEM
   ↳ 8/19/16 → Results: ~~lots of small purple colonies~~ dull purple colonies, <100. → a few white colonies
2) 8/18/16 pAR LacI TS Purp LAA AK3 DH10 iGEM
   ↳ 8/19/16 → Results: ~~<100 colonies, purple, & more dull than~~ many small, purple colonies
3) 8/18/16 R ClpXP CI in AK3
   ↳ Digested (22 µL DNA, 2 µL X, 1 µL P, 14.8 dH_2O)
   ↳ 8/19/16 → Results: lots of colonies, various sizes, some are more clustered than others
4) 8/18/16 puc19 control
   ↳ 8/19/16 → Results: no growth, part of agar gone?

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TITLE 10/13/2016 Inoculation **PROJECT**

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1. pAR LacI GFP LAA ClpXPCI
2. pAR LacI GFP Ø
3. pAR LacI GFP DAS
4. pAR LacI GFP LAA

} constructs

cells { DH10, Keio Wild, Keio ClpP

inducing IPTG levels { no IPTG, 10 uM IPTG, 100 uM IPTG, 1 mM IPTG

not enough cells w/ constructs

10/15/2016 Inoculation

	DH10 cells	Keio Wild	Keio ClpP	
pAR LacI GFP Ø	x3	x3	x3	in triplicates, induced with 0 uM IPTG and 100 uM IPTG
pAR LacI GFP DAS	x3	x3	x3	
pAR LacI GFP LAA ClpXPCI	x3	x3	x3	
Controls	x1	x1	x1	x1 Plain LB

Results: cells grew, but did not fluoresce

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Cell Type	Construct	# Tubes	IPTG
DH10	pAR-LacI TSPurple ClpXPCI	3	0uM
	pAR LacI TS Purple DAS ClpXPCI	3	0 uM
	pAR LacI TS Purple LAA ClpXPCI	3	0 uM
	control	1	0uM
Keio Wild	pAR LacI TS Purple ClpXP CI	12	0 uM, 10 uM, 100 uM, 1mM
	pAR LacI TS Purple DAS ClpXPCI	12	
	pAR LacI TS Purple LAA ClpXPCI	12	
	control	1	
Keio ClpP Knockout	pAR LacI TS Purple ClpXPCI	12	0 uM, 10 uM, 100 uM, 1mM
	pAR LacI TS Purple DAS ClpXPCI	12	
	pAR LacI TS Purple LAA ClpXPCI	12	
	control	1	
no cells	plain LB	+ 1	0 uM
		85 tubes	

4 mL ~~plain~~ Kan LB into each tube
140 mL → Kan LB with NO IPTG
100 mL → Kan LB with 10 uM IPTG
100 mL → Kan LB with 100 uM IPTG
100 mL → Kan LB with 1 mM IPTG

3 uL DNA into DNA
↳ take smear of colony from plate, dilute into 50 uL water

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